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Note

A new chemical synthesis of Ascopyrone P from 1,5-anhydro-D-fructose

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Abstract—The naturally occurring antioxidant Ascopyrone P (1,5-anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose, **1**) was prepared from the rare sugar 1,5-anhydro-D-fructose (AF, **3**) in three steps in an overall yield of 36%. Thus, acetylation of **3** afforded the enolone 3,6-di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-3-en-2-ulopyranose (**4**), which could be isomerised to 2,6-di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-1-ene-3-ulose (**6**). Deacetylation of **6** under mild conditions gave crystalline Ascopyrone P (**1**).

Keywords: Ascopyrone P; 1,5-Anhydro-D-fructose; Antioxidant; Synthesis

1,5-Anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose (**1**), Ascopyrone P (APP), is a metabolite from fungi[1,2] with antioxidant and antibacterial activities[2,3] as well as antibrowning properties.[4] APP (**1**) can only be found in sparse amounts in nature where it is formed predominantly from breakdown of 1,5-anhydro-D-fructose (AF).[5] It has previously been prepared by pyrolysis of cellulose and other natural occurring polysaccharides in about 1.5% yield.[6] A chemical synthesis of **1** was reported recently, which started from 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol (tetra-*O*-acetyl-2-hydroxy-D-glucal, **2**) and gave **1** in an overall yield of 15%.[7] Compound **2** was prepared from D-glucose in three steps[8,9] (Fig. 1). Likewise, an enzymatic preparation of APP from starch has recently been patented.[10]

In view of the interesting properties of APP and the lack of efficient synthetic procedures for its preparation, we undertook the investigation of synthesis of APP from 1,5-anhydro-D-fructose (**3**). This rare sugar is formed by degradation of starch by α-1,4-glucan lyase (EC 4.2.1.13)[11,12] and can now be produced enzymatically from starch[13] but has not lead to AF as a commercial product. The chemistry and biochemistry of AF has recently been reviewed,[14] and we have now developed and improved chemical synthesis of 1,5-anhydro-D-fructose.[15]

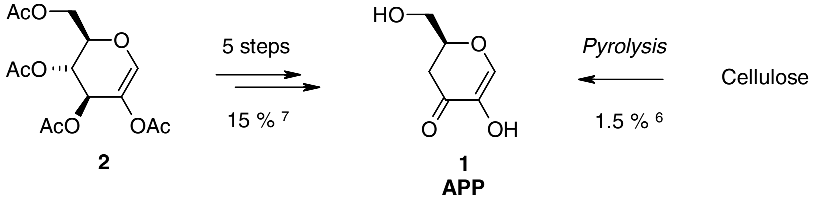

Figure 1.

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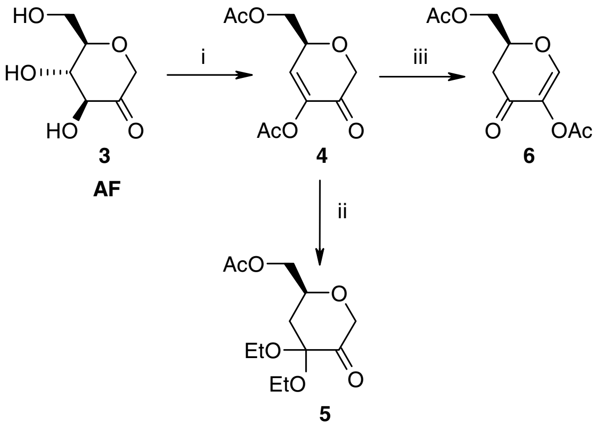


Scheme 1. Reagents and conditions: (i) H_2O, 30 equiv, 2 h, then Ac_2O, pyridine;[19] (ii) DIPEA, EtOH; (iii) Et_3N, CH_2Cl_2.

In our ongoing investigations of using AF as a starting material for new organic compounds,[14,16,17] we envisioned that acetylated APP (**6**) might be obtained by rearrangement of the acetylated enolone **4**, which can be obtained directly from AF (**3**) by acetylation (Scheme 1). 1,5-Anhydro-D-fructose exists as a mixture of a monomer, the hydrated ketone and two stereoisomeric dimeric forms[14] and acetylation under normal conditions (Ac_2O, pyridine) gave, in addition to the enolone **4**, a mixture of acetylated dimers.[14,18] The method was improved by pretreating **3** with an excess of water to convert all forms of AF into the hydrated form before acetylation in pyridine to give **4** as the only compound.[16,19]

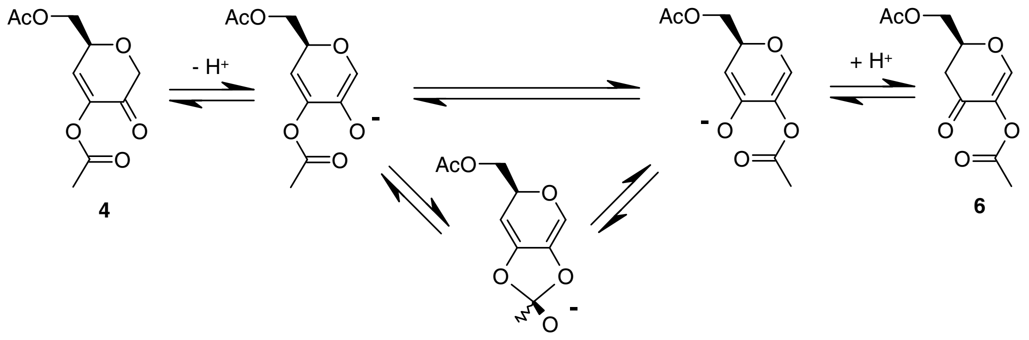
To perform the rearrangement of **4** into the acetylated APP (**6**) secondary and tertiary amines were tested both in a polar as well as in a non-polar solvent. In ethanol, *N*,*N*-diisopropyl-*N*-ethyl amine (DIPEA) surprisingly gave **5**, a diethyl ketal of a 3-oxo-compound, as a mixture with starting material (4:1), whereas the same amine in dichloromethane gave the rearranged product **6** together with starting material (3.5:1). Formation of the ketal **5** in ethanol might be explained by a faster selective deacetylation of the enolic acetate to give formally the 3-keto derivative, which during the acidic work-up reacts with the solvent.

Other amines like piperidine and triethylamine were investigated and they gave similar results. In dichloromethane, piperidine afforded the fastest conversion but triethylamine gave the relative best yield with a ratio of **4**:**6** being about 1:4. The rearrangement proved to be faster when using a secondary amine but the amount of decomposed material was also greater as **6** is sensitive to base.

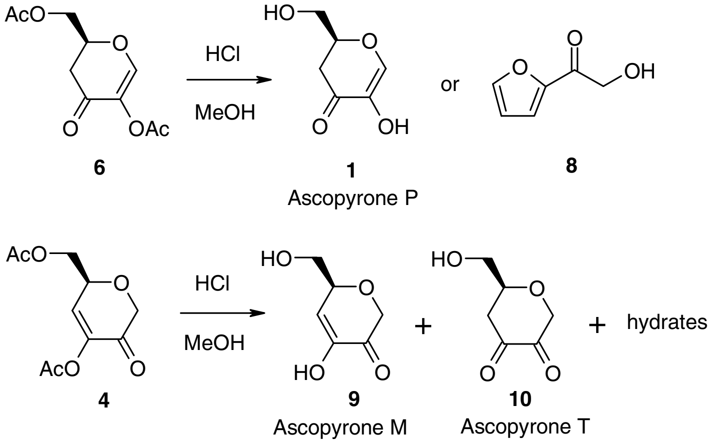
Using a tertiary amine gave a slower reaction but an improved yield. The equilibrium between **4** and **6** was shifted continuously towards **6** but the product was also degraded under the reaction conditions. Allowing the reaction to stir overnight gave a higher ratio of the desired product but also a lower overall yield of both substrates. The reaction conditions were optimised to quench the mixture even if some starting material still was left. The driving force for the rearrangement is formation of a more stable conjugate system in **6** ($–C_3(O)–C_2(OAc)=C_1–O–$) than in **4** ($–C_4=C_3(OAc)–C_2(O)–C_1$) (Scheme 2). The acetylated APP (**6**) could be purified by flash chromatography of the mixture and was isolated in 59% yield.

Finally, deacetylation of **6** to give Ascopyrone P was performed under acidic conditions because the compound is base sensitive; however, too strongly acidic conditions resulted in an almost quantitative formation of 2-hydroxy-1-(2-furyl)ethanone (**8**)[20] (Scheme 3). Dry methanol containing 0.01–0.05% dry HCl resulted in a clean deacetylation to give the crystalline Acopyrone P (**1**) in 77% yield after column chromatography (Scheme 5).

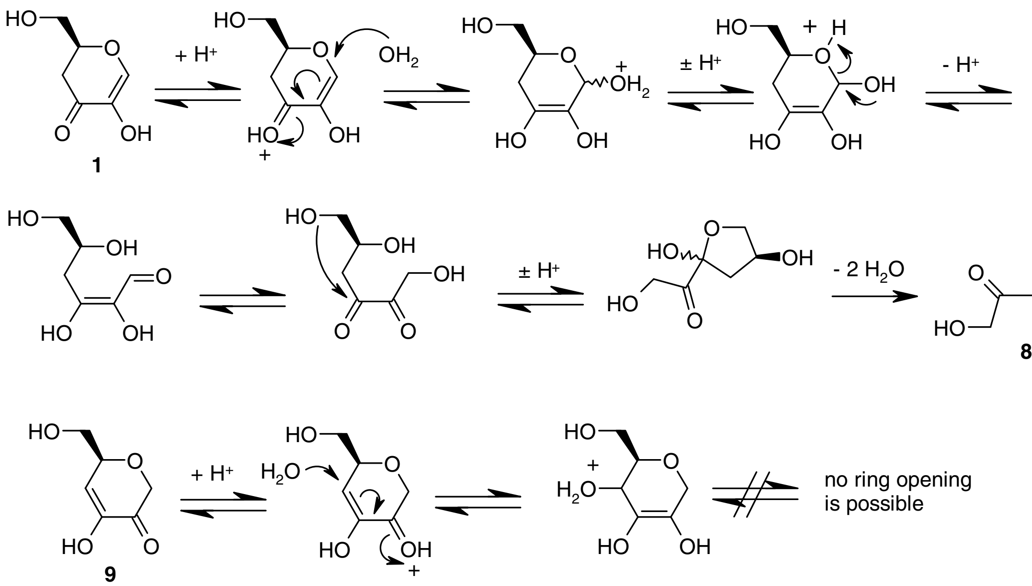
The contrast in stability between **4** and **6** towards acid is striking. The enolone **4** could be deacetylated using strong aqueous acid (4 M HCl) giving the Ascopyrone M (**9**) and corresponding isomers thereof,[7] whereas treatment of **6** with 1% HCl in methanol exclusively gave the furan **8** in 68% yield (Scheme 3). The formation of **8** probably involves, the addition of water to the conjugated system in **6** leading to a free sugar, which



Scheme 2. Mechanism of the rearrangement of enolone **4** to acetylated APP (**6**).



Scheme 3. Deacetylation of **6** compared to **4**.[7]



Scheme 4. Degradation pathways of **1** and **9** under acidic conditions.

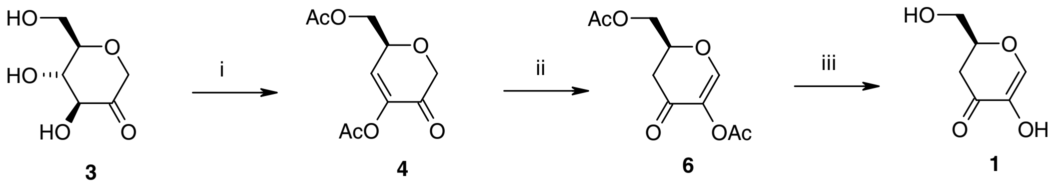
after opening to the aldehyde can be transformed into the furan as suggested in Scheme 4. A similar conjugate addition of water to the deacetylated enolone **9** will not lead to further transformations. It is well known that sugars are degraded under acidic conditions leading to formation of aromatic compounds among which are furan derivatives.[21] The 2′-furyl hydroxymethyl ketone (**8**) has previously been identified in acid degradation of hexoses[21,22] via unsaturated keto sugars.[23]

In conclusion, we have shown that 1,5-anhydro-D-fructose (**3**) can be transformed into Ascopyrone P in three steps in an overall yield of 36% (Scheme 5). Compared to the previous published synthesis, consisting of five steps and starting from the advanced intermediate 2,3,4,6-tetra-*O*-acetyl-1,5-anhydro-D-*arabino*-hex-1-enitol (**2**)[7] or eight steps counting from D-glucose, the existing synthesis has been shortened by five steps if one considers D-glucose to be the starting material. In addition, the overall yield has been improved almost threefold from approximately 13% (from D-glucose) to 36%.

1. Experimental

1.1. General methods

TLC was performed on Merck 60 F254 pre-coated silica plates. Spots were detected by spraying with a solution of *Cemol*: 1.5% $NH_4Mo_2O_2$, 1% Ce(IV)SO_4 and 10% H_2SO_4, followed by heating. For flash chromatography Silica Gel 60, particle size 40–63 µ (Merck) was used. The following solvent systems were used: (A) EtOAc/heptane 1:1, (B) EtOAc/hexane 4:1, (C)



Scheme 5. Reagents and conditions: (i) H_2O, 30 equiv, 2 h, then Ac_2O 15 equiv, pyridine, 80%; (ii) Et_3N (1.5 equiv), CH_2Cl_2, 59%; (iii) CH_3OH/dry HCl (0.01%), rt, 20 h, 77%.

EtOAc/heptane/CH_2Cl_2 1:3:3, (D) CH_2Cl_2/CH_3OH 12:1. Optical rotations were measured on a Perkin–Elmer 241 polarimeter, and the concentrations are given in units of g 100 mL^{-1}. $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded on Varian Mercury 300. Chemical shifts (δ) are reported in ppm using the solvent residues as internal standards: $CDCl_3$ 7.26 (^1H), 77.16 (^{13}C); CD_3OD 3.31 (^1H); 49.0 (^{13}C).[24] Peak multiplicity is reported as s (singlet), d (doublet), t (triplet), m (multiplet) and b (broad). Melting points are uncorrected. CH_3OH was dried over 3 Å molecular sieves, Et_3N was dried over solid KOH, pyridine was dried over solid KOH, CH_2Cl_2 was dried over 4 Å molecular sieves and *N*,*N*-diisopropyl-*N*-ethyl-amine (DIPEA) was dried over 4 Å molecular sieves.

1.2. 3,6-Di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-3-en-2-ulopyranose (4)[19]

1,5-Anhydro-D-fructose (1.04 g, 6.2 mmol, 1 equiv) was mixed with H_2O (3.4 mL, 30 equiv) and the suspension was stirred for 2 h at 25 °C. Pyridine (50 mL) was then added and the solution was cooled to 0 °C. Ac_2O (26.24 mL, 45 equiv) was added and the mixture was stirred for 2 h at rt followed by addition of H_2O (100 mL) and CH_2Cl_2 (100 mL). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (25 mL). The combined organic phases were washed with aq HCl (1 M), satd aq $NaHCO_3$ and H_2O (50 mL), dried ($MgSO_4$), filtered and concentrated in vacuo to give a brown syrup. The residue was co-concentrated with toluene (3 × 10 mL), to give a coloured syrup (1.26 g, 90%), which contained a trace of pyridine. The residue was purified by flash chromatography (1:1, EtOAc/heptane) to yield **4** as a colourless syrup (1.09 g, 78%); $[\alpha]_D^{20}$ −42.8 (*c* 1.7, $CHCl_3$); $R_f = 0.42$ (solvent B); ^1H NMR ($CDCl_3$): δ 6.58 (d, 1H, $J_{4,5}$ 2.9 Hz, H-4), 4.77 (m, 1H, H-5), 4.39 (d, 1H, $J_{1a,1b}$ 15.6 Hz, H-1a), 4.37 (dd, 1H, $J_{6a,6b}$ 13.9, $J_{6a,5}$ 7.8 Hz, H-6a), 4.20 (dd, 1H, $J_{6b\text{–}5}$ 3.9 Hz, H-6b), 4.19 (d, 1H, H-1b), 2.18 (s, 3H, $C\mathit{H}_3CO$), 2.02 (s, 3H, $C\mathit{H}_3CO$); ^{13}C NMR ($CDCl_3$): δ 187.8 (C-2), 170.6, 168.1 ($CH_3\mathit{C}O$), 143.9 (C-3), 133.1 (C-4), 72.8 (C-5), 71.5 (C-1), 64.5 (C-6), 20.5, 20.4 ($\mathit{C}H_3CO$). Anal Calcd for $C_{10}H_{12}O_6$: C, 52.63; H, 5.30. Found: C, 52.12; H, 5.40.

1.3. 6-*O*-Acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hexo-2,3-diulo-pyranose 3,3-di-ethyl acetal (5)

Acetylated enolone **4** (0.612 g, 2.7 mmol, 1 equiv) was dissolved in EtOH (15 mL) and DIPEA (0.693 g/ca. 0.77 mL, 2.0 equiv) and the solution was stirred overnight at rt. The solution was concentrated in vacuo to a residue to which CH_2Cl_2 was added together with aq HCl (1 M). The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic phases were washed with aq HCl (1 M), satd aq $NaHCO_3$, water and dried ($MgSO_4$), filtered and concentrated in vacuo to yield a brown syrup, which consisted of starting material and **5** in about 1:1 ratio. Flash chromatography (1:3:3, EtOAc/heptane/CH_2Cl_2) gave **5** as a colourless syrup (0.330 g, 47% yield); $R_f = 0.26$ (solvent C); ^1H NMR ($CDCl_3$): δ 4.14 (d, 1H, $J_{1a,1b}$ 16.0 Hz, H-1a), 4.06 (dd, 1H, $J_{6a,6b}$ 13.8, $J_{6a,5}$ 7.2 Hz, H-6a), 3.99 (1H, d, $J_{6b\text{–}5}$ 3.8 Hz, H-6b), 3.98–3.90 (m, 4H, 2 × $C\mathit{H}_2O$), 3.74 (d, 1H, H-1b), 3.68 (dddd, 1H, $J_{5\text{–}4ax}$ 14.1, $J_{5\text{–}4eq}$ 4.5 Hz, H-5), 2.82 (dd, 1H, $J_{4ax\text{–}4eq}$ 16.7 Hz, H-4ax), 2.59 (dd, 1H, H-4eq), 2.03 (3H, s, $C\mathit{H}_3CO$), 1.13–1.06 (6H, m, 2 × $C\mathit{H}_3CH_2$); ^{13}C NMR ($CDCl_3$): δ 196.1 (C-2), 169.8 ($CH_3\mathit{C}O$), 102.4 (C-3), 78.2 (C-5), 72.7 (C-1), 65.2 (C-6), 59.5, 59.2 ($\mathit{C}H_2O$), 40.4 (C-4), 20.8 ($\mathit{C}H_3CO$), 12.2, 11.9 ($\mathit{C}H_3CH_2$).

1.4. 2,6-Di-*O*-acetyl-1,5-anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose (6)

To a solution of the enolone **4** (0.672 g, 2.9 mmol, 1 equiv) in CH_2Cl_2 (20 mL) was added Et_3N (0.38 mL, 1.0 equiv) at 0 °C. The mixture was warmed to rt and stirred for 0.5 h, followed by addition of an additional amount of Et_3N (0.19 mL, 0.5 equiv). After stirring for 16 h at ambient temperature aq HCl (10 mL) was added and the phases were separated and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with aq HCl (1 M) and satd aq $NaHCO_3$, water, dried ($MgSO_4$), filtered and concentrated in vacuo to a brown syrup (0.484 g, 72%), which consisted of a mixture of **4** and **6**. The isomers were separated by flash chromatography (1:3:3, EtOAc/heptane/CH_2Cl_2) and **6** was isolated as a clear syrup (0.397 g, 59%); $[\alpha]_D^{20}$ 48.5 (*c* 1, $CHCl_3$); $R_f = 0.27$ (solvent C); ^1H NMR ($CDCl_3$): δ 7.36 (s, 1H, H-1), 4.71 (m, 1H, H-5), 4.33 (dd, 1H, $J_{6a,6b}$

13.3, J_{6a-5} 7.2 Hz, H-6a), 4.27 (dd, 1H, J_{6b-5} 4.6 Hz, H-6b), 2.78 (dd, 1H, $J_{4ax,4eq}$ 17.0, J_{4ax-5} 14.1 Hz, H-4ax), 2.52 (dd, 1H, J_{4eq-5} 3.7 Hz, H-4eq), 2.10 (s, 3H, CH_3CO), 2.04 (s, 3H, CH'_3CO); ^{13}C NMR ($CDCl_3$): δ 183.9 (C-3), 170.3, 168.6 (CH_3CO), 155.1 (C-1), 131.8 (C-2), 77.4 (C-5), 63.9 (C-6), 37.3 (C-4), 20.6, 20.1. Anal Calcd for $C_{10}H_{12}O_6$: C, 52.63; H, 5.30. Found: C, 52.28; H, 5.42.

1.5. 1,5-Anhydro-4-deoxy-D-*glycero*-hex-1-en-3-ulose (Ascopyrone P) (1)

To acetylated APP (**6**, 0.215 g, 0.94 mmol) was added CH_3OH (10 mL) containing 0.01% dry HCl (made by addition of 0.12 mL AcCl to 100 mL CH_3OH) at ambient temperature and the mixture was left overnight. Then, basic ion-exchange resin (Amberlite IR-400) was added and the mixture was stirred briefly, filtered and concentrated in vacuo. Flash chromatography (12:1, CH_2Cl_2/CH_3OH; $R_f = 0.32$) gave **1** as a colourless crystalline compound (0.104 g, 77%); Mp 92–94 °C (lit.[6] 90–95 °C; lit.[7] 98.5–99 °C); $[\alpha]_D^{20}$ +143.0 (*c* 1.0, H_2O) (lit.[7] +139.5 (*c* 1.0, H_2O), lit.[6] +155 (*c* 1.1 H_2O)); $R_f = 0.32$ (D); ^1H NMR (D_2O): δ 7.51 (s, 1H, H-1), 4.54 (m, 1H, H-5), 3.82–3.79 (m, 2H, H-6a+b), 2.82 (dd, 1H, $J_{4ax,4eq}$ 14.7, $J_{4ax,5}$ 4.8 Hz, H-4ax), 2.50 (dd, 1H, $J_{4eq,5}$ 5.1 Hz, H-4eq); ^{13}C NMR (D_2O): δ 190.1 (C-3), 150.1 (C-1), 133.6 (C-2), 78.4 (C-5), 61.3 (C-6), 35.1 (C-4). Anal Calcd for $C_6H_8O_4$: C, 50.00; H, 5.59. Found: C, 49.80, H, 5.68.

1.6. 2-Hydroxy-1-(2-furyl)ethanone (8)

To the acetylated APP (**6**) (0.302 g, 1.3 mmol, 1 equiv) was added CH_3OH (10 mL) containing 1.0% of dry HCl (made by addition of 1.2 mL AcCl to 10 mL CH_3OH) and stirred at ambient temperature for 16 h. Then basic ion-exchange resin (Amberlite IR-400) was added and the mixture was stirred briefly, filtered and concentrated in vacuo. The residue was purified by flash chromatography (16:1, CH_2Cl_2/CH_3OH; $R_f = 0.50$) to give **8** as a crystalline compound (0.113 g, 68%); Mp 83–85 °C (lit.[20] 87–88 °C). The NMR data were consistent with that reported in the literature.[20]

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